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Immunology

Turning (*Ir* gene) low responders into high responders by antibody manipulation of the developing immune system

(idiotypic network/T-cell receptors/maternal influences)

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ABSTRACT **The ability of helper T cells directed against trinitrophenyl-modified syngeneic spleen cells to recognize low-hapten densities on target cells is under major histocompatibility complex-linked *Ir* gene control. Thus, BALB/c (H-2^d) mice are low responders while H-2 congenic BALB.C3H (H-2^k) mice are high responders. Immunization of adult BALB/c mice with the monoclonal antibody F6(51), directed to shared idiotopes by anti-trinitrophenyl antibodies and clonal receptors on anti-trinitrophenyl-self helper T cells, leads to the production of high titers of circulating idiotype, has no influence on helper T cell idiotypic profiles, but shifts to a high-responder phenotype the ability of helper T cells to recognize low-hapten densities. These effects on *Ir* gene phenotype are even more striking in untreated progenies from F6(51)-immunized BALB/c females, which are better responders than genetically high-responder BALB.C3H mice, although completely different in the expression of the F6(51)-defined clonotype. The general significance of these findings on *Ir* gene-directed T-cell repertoire selection is discussed, for they constitute formal evidence against antigen-presentation as a mechanism of *Ir* gene effects and strong support for the importance of maternal influences on the development of T-cell repertoires.**

T-cell repertoires are believed to be principally selected in the thymus upon confrontation of newly expressed clonal receptors with self MHC (major histocompatibility complex) antigens (1–4). Current views consider intrathymic selection to result simultaneously in "self-restriction" and in absence of "self-reactivity" among mature T cells (5). The individuality of the T-cell compartment of immune systems is then primarily determined by MHC polymorphisms, rather than by putative polymorphisms in T-cell receptor (TCR) genes. For those who consider MHC-linked *Ir* genes as solely affecting T-cell repertoires, high and low responsiveness to antigens is exclusively determined in the thymus (6).

Independent evidence has been accumulating for the importance of immunoglobulin (Ig) genes and proteins in the selection of mature T-cell repertoires (7, 8). These notions imply that extensive selection operates at the periphery among postthymic T cells that further modulates the repertoires initially selected in the thymus and is made possible by the population dynamics in this cell pool (9). Extensions of these views would consider that high/low-responder phenotypes are the final result of MHC- *and* Ig-dependent selection of peripheral repertoires. It follows that high/low responsiveness would not be a clonal property (high or low frequency of T-cell clones emerging from the thymus with a given specificity) necessarily resulting from the expression of a given MHC gene, but rather a systemic characteristic of an immune system organized around interactions among T- and B-lymphocyte receptors and self (MHC) components (10). It could then be possible to modulate *Ir* gene phenotypes by idiotypic manipulation of normal individuals and thus interfere with the Ig-dependent part of T-cell repertoire selection.

We report here one example where this was accomplished. Mice of the low-responder (LR) MHC haplotype are turned into high responders (HR) if their idiotypic repertoires are altered during neonatal development. Interestingly, there is no correlation between the idiotypes manipulated and the clonotypic markers expressed by high- or low-responder T cells. It is thus demonstrated that Ig-dependent selection is not only important in establishing TCR idiotypic profiles but in the antigen/MHC specificity repertoires as well.

MATERIALS AND METHODS

Mice. Mice of the strains BALB/c and BALB.C3H were used between 10 and 16 weeks of age.

Monoclonal Antibodies and Idiotype Assays. The MOPC 460 2,4,6-trinitrophenyl (TNP)-specific myeloma protein was purified from ascitic fluids by affinity chromatography. F6(51) is an anti-idiotypic antibody against MOPC 460 (11) and was purified by protein A affinity from ascitic fluids. Antibody determinations were done as described (12) by a hemagglutination assay using TNP-derivatized sheep erythrocytes or erythrocytes coated with MOPC 460 or F6(51) by the chromium chloride method.

Immunizations. Ab_3 mice were prepared as described (13) by immunization with 50 μg of F6(51) copolymerized with keyhole limpet hemocyanin by glutaraldehyde, in complete Freund's adjuvant, followed 2 and 7 weeks later by similar injections in incomplete Freund's adjuvant. Ab_3 females were mated just after the last immunization and used for experiments 3 months later, together with their progenies.

Cell Cultures. Lymphoid cell suspensions were prepared as described (14). B-lymphocyte responses were induced by coculture with specific helper T cells (T_h) in 0.2-ml cultures in microtiter plates (see *Results*). The culture medium was RPMI 1640 supplemented with a growth-supporting, nonstimulatory selected batch of fetal calf serum (GIBCO, 133) at 10%, 50 μM 2-mercaptoethanol, 10 mM Hepes, and antibiotics. TNP and fluorescein isothiocyanate (FITC)-derivatized spleen cells were prepared as previously described (14) after red cell removal.

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Abbreviations: MHC, major histocompatibility complex; TCR, T-cell receptor; LR, low responder; HR, high responder; TNP, 2,4,6-trinitrophenyl; FITC, fluorescein isothiocyanate; T_h, helper T cells; PFC, plaque-forming cells; Ab_3, animals immunized with F6(51) anti-idiotype antibodies as adults.

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Helper Cell Lines. T_h lines specific for TNP- or FITC-syngeneic spleen cells were prepared and maintained *in vitro* as described (14). T_h activity was quantitated upon coculture of a T_h titration with hapten-derivatized syngeneic spleen cells and measuring the number of IgM-secreting cells in the staphylococcal protein A plaque assay (15) 4–6 days later.

Limiting-Dilution Assay. Nylon wool-purified spleen T cells were incubated 4 hr with Con A and thereafter washed in methyl α-D-mannoside-containing balanced salt solution. Titrated numbers of induced T cells were cultured with 4×10^4 either normal or TNP-derivatized syngeneic spleen cells (TNP_5 or $TNP_{0.05}$), and the plaque-forming cell response in each culture was evaluated 5 days later. For each experimental point, 36–48 identical cultures were set up, and frequency determinations were calculated from a minimum of four points (cell concentrations).

RESULTS

MHC-Linked Genes Determine the Ability of T_h Cells to Recognize Low Densities of Hapten on Syngeneic Cells. In similarity with experimental systems analyzing cytolytic T-cell responses (16), we have originally described that mouse strains differ sharply in the ability of T_h cells to respond to syngeneic spleen cells derivatized with low hapten densities (17). Thus, T_h cells from CBA and DBA/2 mice, while equally competent to interact with "self" cells modified with high concentrations of the haptens TNP, 3-(*p*-sulfophenyldiazo)-4-hydroxyphenyl (SP), and FITC, are HRs and LRs, respectively, when the same haptens are coupled at low density to syngeneic cells. More recently, using H-2 recombinant strains on the C57BL/10 background, we have mapped the genes controlling this phenotype to the I-A region of MHC (18, 19). The same *Ir* gene effect is observed on the BALB/c background, as shown in Fig. 1. Thus, BALB/c and the H-2 congenic BALB.C3H mice both produce T_h cells capable of recognizing TNP modifications of syngeneic spleen cells at high hapten concentrations, but only $H\text{-}2^k$ mice produce T_h cells responding to sparsely hapten-derivatized "self."

Untreated LR Mice Born from Idiotypically Manipulated Mothers Are Phenotypically HRs. In previous experiments we had detected the expression by BALB/c anti-TNP-self T_h cells of an idiotypic determinant, characterized by the anti-idiotypic antibody F6(51) and also carried by the BALB/c anti-TNP myeloma protein MOPC 460 (8). We had shown that T_h cell idiotopes are present on TCR molecules (20) and that the expression of such clonotype is controlled both by MHC- and IgH-linked genes (18). Furthermore, evidence was provided for the "learning" of this T_h cell clonotype from the B cell/antibody compartment (8) in the first 3–4 weeks of life (21). This Ig-dependence of T-cell repertoire selection appeared, therefore, to be a suitable tool to explore the impact of idiotypic interactions on the establishment of MHC-linked *Ir* gene phenotypes. In essence, we have repeated classical experiments of anti-idiotype priming (Ab_3 mice) (22, 23), but rather than simply analyzing idiotypic repertoires in these animals, as done before (12, 13, 22–24), we have also investigated whether or not alterations in components of an idiotypic network could have consequences upon the overall reactivities of the individual, namely HR or LR phenotypes.

Fig. 2 shows the essential results of these experiments. Three groups of BALB/c mice were tested for the ability of their T_h cells to recognize high and low densities of TNP on syngeneic cells—normal untreated mice, animals immunized with F6(51) anti-idiotypic antibodies as adults (Ab_3), and untreated individuals born from Ab_3 females. As can be seen, BALB/c T_h cells fail to recognize low-hapten modification of self, whereas Ab_3 mice produce T_h cells that are indistinguishable from those recovered in HR BALB.C3H mice (see Fig. 1). Strikingly, Ab_3 progenies fail altogether to discriminate low and high density of TNP-syngeneic cells. In other words, T_h cells from Ab_3 progenies respond to low-hapten densities as well as T_h cells from normal mice respond to high densities of TNP, which makes them "higher" responders than the prototype $H\text{-}2^k$ HR strains (see also refs. 17 and 18).

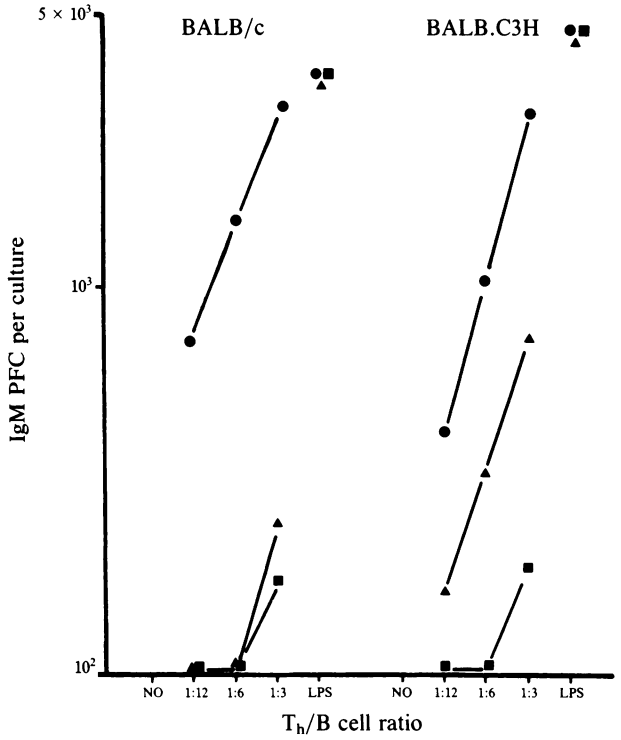

FIG. 1. *Ir* gene effects in the response to TNP-modified self. Titrated numbers of specific syngeneic anti-TNP-BALB/c or anti-TNP-BALB.C3H helper cell lines, as indicated, were mixed with 4×10^4 responding normal (■), high-TNP_5 (●), or low-$TNP_{0.05}$ (▲)-derivatized BALB/c or BALB.C3H spleen cells, and the cooperative IgM plaque-forming cell (PFC) responses were measured 4 days later.

As also shown in Fig. 2, idiotypic treatments have very selective consequences on the ability of T_h cells to recognize low hapten densities. Thus, all three groups of mice are comparable in their LR phenotype to self-modifications with a related hapten (FITC). Furthermore, parallel groups of BALB/c animals, similarly immunized with antibodies other than F6(51), behaved as normal LR BALB/c mice (ref. 25 and C.M.-A., unpublished observations). This also excludes trivial effects of adjuvants and immunization protocols.

We have attempted to correlate the ability or failure of Ab_3 progenies versus normal BALB/c mice to recognize low hapten densities with the frequency at which these T_h cell specificities occur in untreated individuals before antigen-priming and *in vitro* enrichment. Thus, it could be argued that the effects observed were due to altered regulation of antigen-specific immune responses, rather than consequences of true network interactions selecting repertoires before antigenic challenges. Results shown in Table 1 clearly demonstrate the influence of idiotypic manipulation on the available repertoire of T_h cells recognizing high- and low-hapten densities. While the total anti-TNP-self T_h cell frequencies remain constant (which agrees with comparable responses of T_h cells in both cases to high-TNP-self, see Fig. 2), Ab_3 progenies contain exclusively high-affinity T_h cells (able to recognize low hapten densities) in contrast with normal mice whose repertoire predominantly contains low-affinity cells with this specificity.

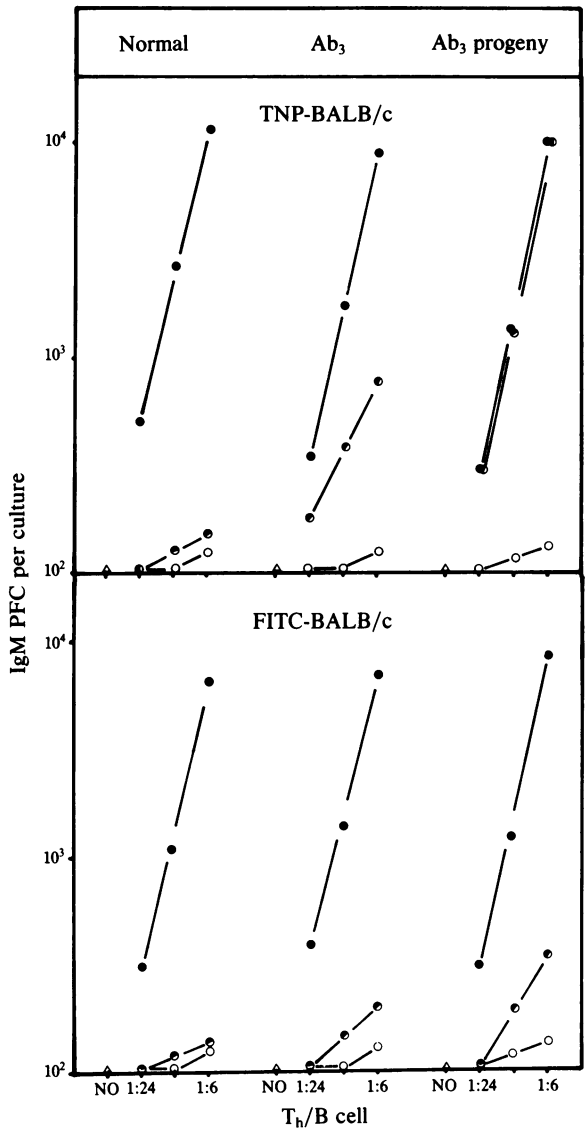

FIG. 2. HR phenotype of idiotypically manipulated BALB/c mice. Helper cell lines specific for TNP- or FITC-BALB/c were derived from BALB/c mice—either normal, Ab_3, or untreated progenies from Ab_3 females. Mice used as T_h cell donors had circulating M460 idiotypic titers of 1:2, 1:8000, and 1:4, respectively. Helper cell lines were tested for helper activity upon coculture with normal (○), TNP_5 (●), and $TNP_{0.05}$ (◑) or normal (○) $FITC_{1.0}$ (●), and FITC 0.01 (◑)-BALB/c derivatized spleen cells as indicated. The IgM PFC response was evaluated 4 days after the initiation of the culture.

Lack of Correlation Between *Ir* Gene Phenotype and T_h Cell Clonotype Expression. The observations reported here appear to result from complex interactions that define global or systemic, rather than clonal, properties of the immune system (10). This is more clearly suggested by the observation that the shift in *Ir* gene phenotype is achieved by manipulations leading to increased expression of an idiotype that is, paradoxically, the T_h clonotype of LR mice. As illustrated in Fig. 3, up to 75% of TNP-self-specific T_h cells from BALB/c LR mice are inhibited by F6(51) antibodies, whereas no significant inhibition is obtained with HR BALB.C3H T_h cells. Furthermore, T_h cells prepared from Ab_3 BALB/c mice or from their progenies, phenotypically HR, are perfectly comparable to LR BALB/c T_h cells in their expression of this particular clonotype. It is otherwise confirmed (see legend to Fig. 2) that Ab_3 mice do produce greatly enhanced levels of idiotype-positive antibodies in their serum, up to 1000-fold more than untreated BALB/c mice.

Table 1. Frequency of self- or TNP-self-specific T_h cells in unprimed BALB/c mice

Responder cell origin	Treatment of responder spleen cells			
	Self	TNP low density*	TNP high density†	TNP total*
BALB/c	1:24,000	1:18,600	1:15,800	1:6300
Ab_3 BALB/c progeny	1:31,000	1:7800	1:100,000	1:6200

Ratio reported as T_h:total cells.
*Anti-self subtracted.
†Anti-self and anti-TNP low-density subtracted.

DISCUSSION

There was a period when the detection of a recurrent antibody idiotype in specific immune responses was believed to accurately correlate with the presence of a particular heavy-chain variable region gene in the germ line. The possibility of inducing idiotypes "à la carte" (22, 23) and of readily suppressing expression of major germ-line idiotypes (see ref. 26), as well as the lack of correlation between the frequencies of idiotype-positive B-cell clonal precursors and the production of the idiotype in immune responses (see ref. 27), indicated, however, the wide boundaries of potential diversity and the prevalence of somatic mechanisms in the selection of available repertoires. As concerns T cells, most studies have concentrated on the analysis of antigen–MHC specificity and have generally concluded that the MHC antigens had a primary role in selecting T cells (1–6), whereas those analyzing idiotypic repertoires have concluded that idiotypic repertoires were controlled by IgH-linked genes (28). These views imply either that germ-line TCR gene repertoires are polymorphic to a limited degree or else, again, that there are wide boundaries in the germ line for potential diversification. More recent experiments, calling attention to the importance of Ig genes and proteins in the selection of both paratopic (7) and idiotypic (8, 29) peripheral repertoires of T cells, led us to suggest that available T-cell diversity was somatically selected from the expressed germ-line diversity by MHC products (in the thymus) and Ig gene products (in the periphery) (18). It follows that T-cell repertoires and, consequently, *Ir* gene phenotypes are expected to be genetically controlled by (*i*) TCR genes, (*ii*) MHC genes, and (*iii*) Ig genes. While practically all classical evidence has been constructed around the MHC-linked control of *Ir* gene phenotypes, recent evidence does indeed show phenotypic effects of TCR genes (30). Furthermore, the linkage of the control of paratopic repertoires to both MHC and IgH genes has been established (7, 31), as well as the consequences of TCR gene polymorphisms on the idiotypic profiles of peripheral T cells (32).

We have previously shown, at the level of idiotypic repertoires, that T lymphocyte diversity is under control of both MHC- and IgH-linked genes (18). We extend here these observations by the demonstration that, in the absence of detectable alterations in idiotypic profiles, Ig-dependent selective processes have direct consequences in the available paratopic repertoires of T_hs and may alter MHC-linked *Ir* gene phenotypes. We are unaware of experiments that directly address the modulatory influence of Ig-encoding genes in studies of MHC-linked *Ir* genes, but as the TCR gene control of T-cell repertoires is only now established in genetic experiments (30), it would not be surprising if the same modulatory influence will be the case for Ig genes.

The lack of correlation between idiotypic and paratopic specificity (or, at least, affinity) of TCR exemplified here is only surprising for those who consider mature repertoires as

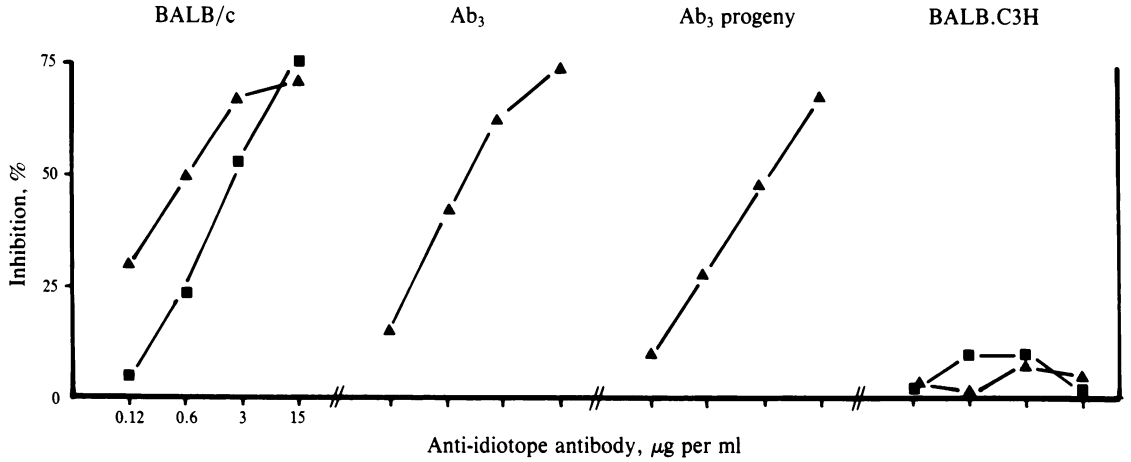

FIG. 3. Idiotype expression by HR and LR T_h cells. Specific anti-TNP-self helper cells (3×10^3 per culture) from BALB.C3H and BALB/c mice—either normal, Ab_3, or progenies from Ab_3 females—were mixed with 5×10^4 responding TNP-derivatized syngeneic cells, and the cooperative IgM PFC responses were measured 4 days later. The cells were cultured either with or without the indicated concentrations (μg/ml) of purified F6(51) antibodies (▲). Results show the percentage of inhibition obtained by the addition of the F6(51) at the start of cultures. Noninhibited control responses were as follows: 8100, 4800, 7200, and 10,700 IgM PFC per culture for BALB.C3H, normal BALB/c, Ab_3 BALB/c, or progenies from Ab_3 BALB/c females, respectively. BALB.C3H and normal BALB/c helper cells (but not from Ab_3 BALB/c or Ab_3 progenies) were also tested for proliferative responses (controls 51,100 and 83,400 cpm per culture, respectively) and their inhibition by F6(51) antibodies (■).

an accumulation of individually selected clones—defined by the expression of a pair of variable region genes—upon interactions with a single class of selecting molecules. Alternatively, if repertoire selection is considered to represent the transitory result of multiple interactions with a variety of molecules—MHC products, antibodies, and other TCRs—that may address either idiotypic aspects, paratopic reactivities, or both, on each individual T cell (10, 33–36), it then becomes evident that T-cell repertoires can only be understood as a unit participating in a larger, systemic organization that defines every immune system. It then follows that full understanding of *Ir* gene effects requires these global perspectives that have thus far been given little attention in contrast with (few) properties of single clones. It also follows from these arguments that both thymic and postthymic selection are expected to contribute in the establishment and maintenance of T-cell repertoires, even if it is likely that for each type of selecting molecules and/or clonal specificity considered, predominance of a particular site and T-cell developmental stage of selection will be found. This could well be at the origin of the current controversies on this topic.

Although our present observations are general and do not explain molecular or cell interaction mechanisms operating in T-cell repertoire selection, it is worth noting that they are at variance with molecular models proposing that all *Ir* gene control of immune responses is mediated by antigen presentation (37). Thus, we show that repertoire alterations (from LR to HR phenotype) are already present *before* antigenic challenge and, therefore, presentation. In addition, TNP-derivatized presenting cells were, in all these experiments, from normal BALB/c LR mice. Finally, if MHC-linked high or low responsiveness were to be exclusively explained on the basis of expression, rates of turnover, and shedding of I-A molecules, or their processing after TNP conjugation, we cannot see how any of these could have been altered by idiotypic manipulation. It thus appears most likely that, at least in this case, high/low responder status is a direct reading of T-cell repertoires. In this context, the determinant influences of the maternal immune system in establishing repertoires of progenies acquire particular relevance. We had previously described this type of influence upon the B-cell compartment of newborns (38), and they are extended here to the T-cell compartment and to later stages in life. By inference, we can expect this fundamental "learning" process to occur with every immune system from the immunological status of the mother at the time of pregnancy, which, in turn, depends on its own ontogeny. That is, the ontogeny of immune systems starts with the ontogeny of the mother's immune system. Interestingly, as shown here, the mother can transfer to progenies a responding phenotype that she herself does not possess, at least to the same extent, and this fact could be of importance in the understanding of familial influences in the inheritance of, for example, MHC-associated pathology.

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